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# Taxonomic position of *Hormaphis similibetulae* Qiao & Zhang, 2004 (Hemiptera, Aphididae): molecular and biological evidences

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## Abstract

The taxonomic position of *Hormaphis similibetulae* Qiao & Zhang, 2004 has been reexamined. The phylogenetic position of *H. similibetulae* was inferred by maximum parsimony, maximum likelihood and Bayesian analyses on the basis of partial nuclear elongation factor-1α and mitochondrial tRNA leucine/cytochrome oxidase II sequences. The results showed that this species fell into the clade of *Hamamelistes* species, occupying a basal position, and was clearly distinct from other *Hormaphis* species. A closer relationship between *H. similibetulae* and *Hamamelistes* species was also revealed by life cycle analysis. Therefore, we conclude that *H. similibetulae* should be transferred to the genus *Hamamelistes* as *Hamamelistes similibetulae* (Qiao & Zhang), **comb. n.**

## Keywords

Hormaphidinae, *Hormaphis similibetulae*, molecular evidence, biological evidence, new combination, China

## Introduction

The aphid tribe Hormaphidini in subfamily Hormaphidinae (Hemiptera: Aphididae) consists of three genera, *Hamamelistes*, *Hormaphis* and *Protohormaphis* (Remaudière and Remaudière 1997). *Hamamelistes* and *Hormaphis* are disjunctively distributed in

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Eurasia and North America (Guo and Qiao 2005), where they are primarily associated with *Hamamelis* and secondarily associated with *Betula*. The taxonomy of these two genera was once in a mess at both the genus and species levels. They are easily confused with one another, and species of the same genus are difficult to distinguish morphologically. This confusion in the taxonomy was due partly to a limitation of diagnostic characteristics and partly to the fact that no combination had been established between different aphid forms on primary and secondary hosts. Distinction of *Hamamelistes* and *Hormaphis* is based mainly upon alatae, galls and life cycles. However, it is not easy to collect all morphs, and the observation of life cycles takes a long time. Molecular studies have shed light on these issues. Based on the mitochondrial cytochrome oxidase II (COII) gene, Aoki et al. (2001) clarified the Japanese *Hamamelistes* species, established the combination between generations on primary and secondary hosts, and elucidated their life cycles. von Dohlen et al. (2002) estimated the phylogeny of Hormaphidini using partial nuclear elongation factor-1α (EF-1α) and mitochondrial tRNA leucine/cytochrome oxidase II (COII) sequences, the monophyly of both *Hamamelistes* and *Hormaphis* was retrieved with strong support.

Qiao and Zhang (2004) described *Hormaphis similibetulae* based on specimens of apterous viviparous females collected from small conical galls on leaves of *Betula albosinensis* in China (Tibet); the specimens were closely related to *Hormaphis betulae* (Mordvilko) but differed from the latter in body color and living habits. In this study, the taxonomic position of *H. similibetulae* was reassessed on the basis of nuclear EF-1α and mitochondrial tRNA/COII sequences. A discussion of life cycles was also included.

## Materials and methods

The samples used in this study and the corresponding collection information are listed in Table 1. Eight species of Hormaphidini, covering all the species of *Hamamelistes* and *Hormaphis* were used as ingroups. Three species of Nipponaphidini were chosen as outgroups because Nipponaphidini is considered the sister group of Hormaphidini based on biological and phylogenetic data (Ghosh 1985, von Dohlen and Moran 2000, Ortiz-Rivas and Martínez-Torres 2010). Voucher specimens were preserved in 75% ethanol and deposited in the National Zoological Museum of China, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

Total genomic DNA was extracted from single aphids preserved in 95% or 100% ethanol using a CTAB protocol modified from Doyle and Doyle (1987). Partial leucine tRNA and the cytochrome oxidase II (COII) gene was amplified with primers 2993+ (Stern 1994) and A3772 (Normark 1996). Sequencing reactions were performed using the corresponding PCR primers from both directions with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and run on an ABI 3730 automated sequencer (Applied Biosystems). Sequences were assembled by Seqman II (DNAStar, Inc., Madison, WI, USA) and verified for protein coding frame-

**Table 1.** Collection information and GenBank accession numbers for aphid samples used in this study.

| Species | Host | Locality | Date | Voucher | EF-1× | tRNA/COII |
|---|---|---|---|---|---|---|
| *Hamamelistes betulinus* (Horvath) | *Betula davurica* | Japan: Yamanashi, Masutomi | 17 Jul. 1998 | 98081 | AF454599* | AF328782* |
| | *Hamamelis japonica* | Japan: Aomori, Temmabayashi | 7 Aug. 1998 | 98132 | AF454596* | AF328775* |
| | *Betula platyphylla* | Japan: Tokyo, Okutamako | 20 May 1999 | 99121 | AF454597* | AF328780* |
| | *Betula platyphylla* | Japan: Hokkaido, Sapporo | 15 Jun. 1999 | 99187 | AF454598* | AF328781* |
| *Hamamelistes kagamii* (Monzen) | *Hamamelis japonica* | Japan: Yamanashi, Masutomi | 17 Jul. 1998 | 98084 | AF454600* | AF328772* |
| | *Betula grossa* | Japan: Yamanashi, Sanjonoyu | 20 May 1999 | 99118 | AF454601* | AF328779* |
| | *Hamamelis japonica* | Japan: Saitama, Shomaru Pass | 8 Jul. 1999 | 99209 | AF454603* | AF328773* |
| | *Hamamelis japonica* | Japan: Saitama, Shomaru Pass | 8 Jul. 1999 | 99220 | AF454602* | AF328774* |
| *Hamamelistes miyabei* (Matsumura) | *Hamamelis japonica* | Japan: Yamanashi, Masutomi | 17 Jul. 1998 | 98086 | AF454595* | AF328771* |
| | *Betula maximowicziana* | Japan: Hokkaido, Sapporo | 5 Sep. 1998 | 98151 | AF454593* | AF328776* |
| | *Betula maximowicziana* | Japan: Gumma, Mt. Akagi | 25 May 1999 | 99146 | AF454594* | AF328777* |
| | *Betula maximowicziana* | Japan: Hokkaido, Sapporo | 15 Jun. 1999 | 99182 | AF454592* | AF328778* |
| *Hamamelistes spinosus* Shimer | *Hamamelis japonica* | USA: Washington, DC | May 1993 | 93-23 | AF454606* | AF328783* |
| | *Betula nigra* | USA: UT, Logan | 28 May 1999 | 99-54 | AF454607* | AF454619* |
| | *Betula nigra* | USA: WI, Madison | 28 Jun. 1999 | 99-57 | AF454608* | None |
| *Hormaphis betulae* (Mordvilko) | *Betula platyphylla* | Japan: Yamanashi, Masutomi | 17 Jul. 1998 | 98078 | AF454609* | None |
| | *Hamamelis japonica* | Japan: Saitama, Shomaru Pass | 21 May 1999 | 99130 | AF454610* | AF454622* |
| | *Betula platyphylla* | Japan: Tokyo, Kazahari Pass | 26 Jul. 1999 | 99224 | AF454611* | AF454623* |
| | *Betula* sp. | China: Jilin, Ji'an | 13 Aug. 2004 | 15214 | DQ493864* | JF730745 |
| *Hormaphis cornu* (Shimer) | *Hamamelis virginiana* | USA: Georgia, Athens | 8 Jun. 1994 | 94-93 | AF454612* | AF454621* |
| *Hormaphis hamamelidis* (Fitch) | *Hamamelis virginiana* | USA: Connecticut, Danielson | 1 Aug. 1998 | 98-05 | AF454613* | AF454620* |
| *Hormaphis similibetulae* Qiao & Zhang | *Betula albosinensis* | China: Tibet, Gongbo'gyamda | 5 Jul. 2002 | 13549 | DQ493849* | JF730746 |
| | *Betula albosinensis* | China: Tibet, Linzhi | 6 Aug. 2003 | 15318 | DQ493866* | JF730747 |
| *Neohormaphis wuyiensis* Qiao & Jiang | *Quercus* sp. | China: Fujian, Mt. Wuyi | 18 Jul. 2003 | 14525 | DQ493858* | JF730748 |
| *Nipponaphis distyliicola* Monzen | *Quercus glauca* | Japan: Shinkiba, Tokyo | 16 Apr. 1999 | 99008 | AF454614* | AF454626* |
| *Thoracaphis quercifoliae* Ghosh | *Quercus* sp. | China: Fujian, Mt. Wuyi | 18 Jul. 2003 | 14526_2 | DQ493851* | JF730749 |

* Sequences from GenBank.

shifts to avoid pseudogenes (Zhang and Hewitt 1996) using Editseq (DNAStar, Inc.). All sequences were deposited in GenBank under the accession numbers JF730745–JF730749. All EF-1α sequences used in this study were downloaded directly from GenBank (for accession numbers see Table 1), and only exons were used for further analysis. Multiple alignments were done with ClustalX 1.83 (Thompson et al. 1997) and then verified manually. Nucleotide composition and pairwise distances based on Kimura's two-parameter model (K2P) (Kimura 1980) of the aligned sequences were calculated using MEGA 4.0 (Tamura et al. 2007).

Phylogenetic reconstructions were conducted by maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses for each single gene and a combined dataset. The partition homogeneity test (Farris et al. 1994) based on 100 replicates of a heuristic search algorithm was performed with PAUP*4.0b10 (Swofford 2002) to examine the incongruence between EF-1α and mtDNA. Unweighted MP and ML analyses were carried out using PAUP*. For ML analysis, the best-fit model of nucleotide substitution was selected for each dataset according to the Akaike information criterion (AIC) in Modeltest 3.7 (Posada and Crandall 1998). Heuristic searches were performed with 1000 (MP) or 100 (ML) random-addition sequences and tree-bisection-reconnection (TBR) branch swapping. Bootstrap (BS) analyses were used to assess the relative robustness of branches of the MP (1000 replicates) and the ML (100 replicates) trees (Felsenstein 1985). Bayesian analysis was conducted using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) based on the model selected by Modeltest 3.7. In the combined analysis, the mitochondrial and nuclear data were partitioned, and a heterogeneous model was used for each gene partition. The parameters of the model were treated as unknown variables with uniform prior probabilities and were estimated during the analysis. Four Markov chains (three heated and one cold) were run, starting from a random tree and proceeding for one million Markov chain Monte Carlo generations, sampling the chains every 100 generations. Two concurrent runs were conducted to verify the results. The first 2500 trees were discarded as burn-in samples, the remaining trees were used to compute a majority-rule consensus tree with posterior probabilities (PP).

## Results and discussion

The final alignments of EF-1α (excluding three introns) and tRNA/COII sequences consisted of 826 and 761 sites, with 131 and 165 parsimony-informative sites, respectively. A single 1- to 2-base-long indel was found in the tRNA. The genetic distance between two distinct samples of *H. similibetulae* was 0 for EF-1α and 0.001 for tRNA/COII. The distances of both genes between *H. similibetulae* and *Hamamelistes* species were much smaller than those between *H. similibetulae* and the other *Hormaphis* species (EF-1α: average of 0.040 and range of 0.038–0.042 to *Hamamelistes*, average of 0.082 and range of 0.078–0.092 to *Hormaphis*; tRNA/COII: average of 0.080 and range of 0.071–0.085 to *Hamamelistes*, average of 0.106 and range of 0.102–0.112 to *Hormaphis*).

For phylogenetic analyses, the partition homogeneity test found no significant conflict between EF-1α and mtDNA ($P$=0.05), indicating that information from both genes could be combined. Combined analysis resulted in similar topology to that obtained in single gene analyses and with higher support for most nodes, so only the combined dataset results were presented. MP analysis yielded eight most parsimonious trees with a length of 611 steps (CI=0.705401, RI=0.845626). ML analysis produced one ML tree based on the optimal model GTR+G selected by AIC in Modeltest 3.7. The 50% majority-rule consensus tree inferred from Bayesian analysis is shown in Fig. 1 and resulted in a topology essentially identical to that obtained in ML analysis, but was different from the strict consensus of MP trees in the position of *H. similibetulae*. All ingroup taxa constituted a monophyletic group with respect to these outgroups and formed two clades. Clade I (100% MP BS, 100% ML BS, 1.00 PP) was comprised of *H. betulae*, *H. cornu*, and *H. hamamelidis*. Clade II (99% MP BS, 99% ML BS, 1.00 PP) consisted of all the *Hamamelistes* species and *H. similibetulae*. Within clade II, two distinct samples of *H. similibetulae* clustered together (100% MP BS, 100% ML BS, 1.00 PP) and were placed as the outermost branch in ML and Bayesian analyses, just as the results based on EF-1α. However, MP analysis revealed the same topology as the mitochondrial analysis: *H. similibetulae* and *Hamamelistes spinosus* were sister groups, although the support value was low (53% BS), and together formed the basal lineage within clade II.

The results of genetic distances and phylogenetic analyses strongly suggested that *H. similibetulae* was more closely related to *Hamamelistes* than to *Hormaphis*. *H. similibetulae* was distinguished by its unique biology, forming galls on leaves of *Betula*. Because of the high morphological similarity with *H. betulae* (Mordvilko), it was placed under the genus *Hormaphis* (Qiao and Zhang 2004). However, the distinction of apterae of *Hamamelistes* and *Hormaphis* from the secondary host *Betula* is very difficult: both of them are aleyrodiform, dorsoventrally compressed, have body segments fused, short antennae with only 2–4 segments, fore and middle legs without tarsi, and hind legs with rudimentary unsegmented tarsi and lack claws. These reductions appear to be related to the organisms' sedentary habits on *Betula* and represent the adaptive convergences selected by their temperate habitat. Although species of both genera migrate between *Hamamelis* and *Betula*, their life cycles are quite different and have proven extremely valuable in distinction (Pergande 1901, von Dohlen and Gill 1989, Aoki and Kurosu 1991, von Dohlen and Stoetzel 1991, Aoki et al. 2001). Firstly, *Hamamelistes* have two-year life cycles due to a long gall phase, while *Hormaphis* complete their life cycles within one year. Secondly, on *Hamamelis*, *Hamamelistes* induce spiny or coral-like galls on leaf or flower buds, whereas *Hormaphis* cause conical galls on the leaves. Lastly, *Hamamelistes* inhabit cockscomb-like or blister-like galls on leaves of *Betula*, but *Hormaphis* live freely on the leaves, not causing any deformation. In China, there is only one species of *Hamamelis*, *H. mollis*, distributed in Sichuan, Hubei, Anhui, Zhejiang, Jiangxi, Hunan and Guangxi Provinces (Zhang and Lu 1995). According to the absence of primary host at high elevations in the Tibetan Plateau, Qiao and Zhang (2004) inferred that *H. similibetulae* was autoecious on *Betula albosinensis*. We agree with their inference, as *Ha. betulinus* and *H. betulae* were also observed living all

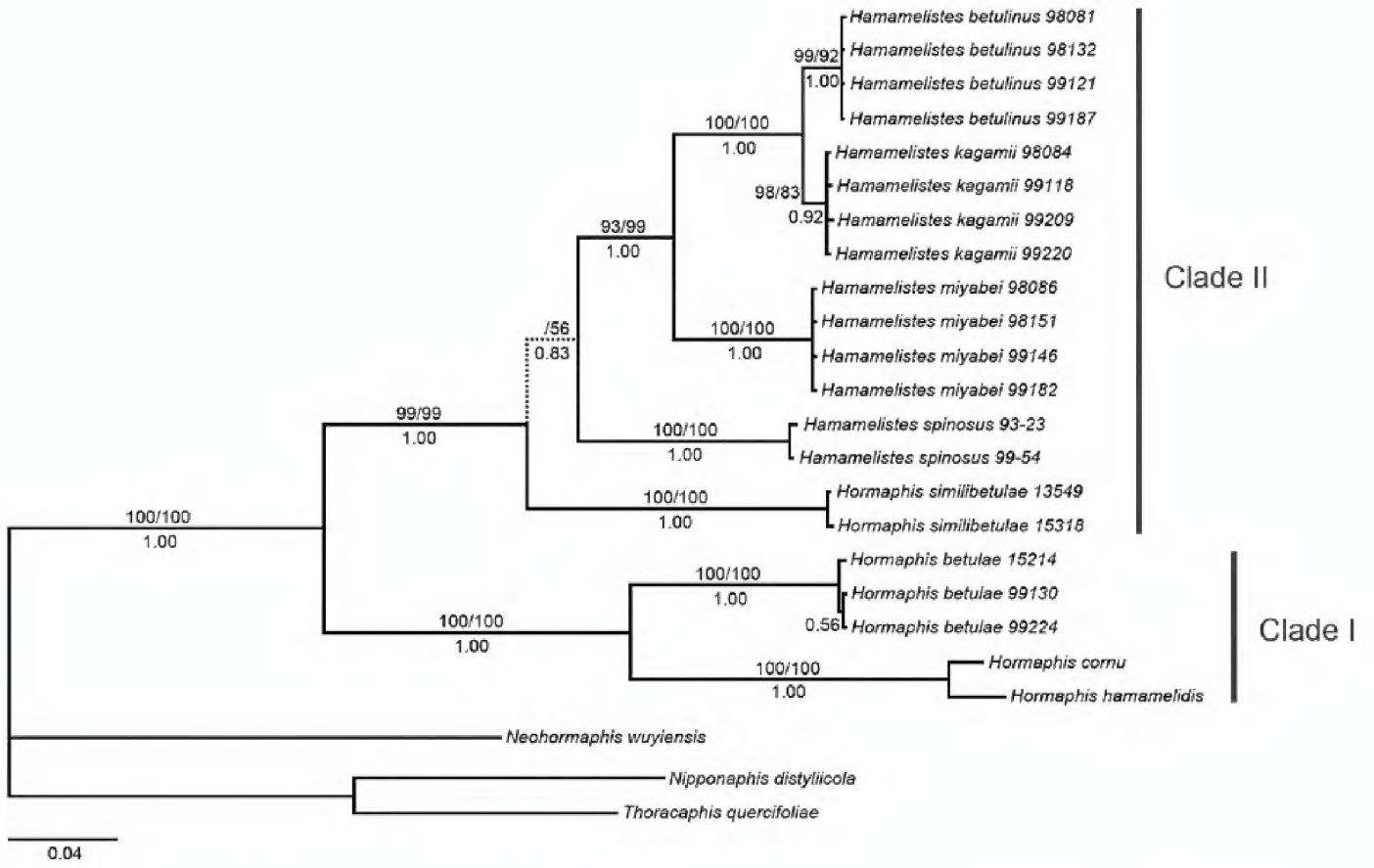


**Figure 1.** Phylogenetic tree reconstructed from the combined dataset of EF-1α and tRNA/COII sequences. The Bayesian topology and branch lengths are shown. Values above the branches are MP and ML bootstrap percentages, respectively, and Bayesian posterior probabilities are shown below the branches. The broken line indicates inconsistent branch.

year round parthenogenetically on *Betula* in Europe due to lack of primary host (Heie 1980). Although the life cycle of *H. similibetulae* requires further research, it appears to be more similar to that of *Hamamelistes* than to that of *Hormaphis*.

## Conclusion

The phylogenetic position of *Hormaphis similibetulae* was inferred by MP, ML and Bayesian analyses on the basis of nuclear EF-1α and mitochondrial tRNA/COII sequences. In all phylogenetic analyses, *H. similibetulae* clustered firmly with *Hamamelistes* and was placed as a basal lineage, clearly differed from other *Hormaphis* species. Life cycle similarities also indicated that *H. similibetulae* was more closely related to *Hamamelistes* species. We therefore conclude that *H. similibetulae* should be transferred to the genus *Hamamelistes* as *Hamamelistes similibetulae* (Qiao & Zhang), comb. n.

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## References

Aoki S, Kurosu U (1991) Host alternation of *Hormaphis betulae* (Homoptera, Aphidoidea). Japanese Journal of Entomology 59:164.

Aoki S, von Dohlen CD, Kurosu U (2001) Revision of the Japanese species of the aphid genus *Hamamelistes* (Hemiptera, Aphididae, Hormaphidinae) based on the mitochondrial DNA sequence data. Entomological Science 4: 59–67.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry Bulletin 9: 11–15.

Farris JS, Källersjö M, Kluge AG, Bult C (1994) Testing significance of incongruence. Cladistics 10: 315–319. doi:10.1111/j.1096-0031.1994.tb00181.x

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. doi:10.2307/2408678

Ghosh AK (1985) Hormaphidinae: distribution, phylogeny and systematics. In: Szelegiewicz H (Ed) Evolution and Biosystematics of Aphids. Proceedings of the International Aphidological Symposium at Jablonna, 1981. Polska Akademia Nauk, Warsaw, 303–336.

Guo K, Qiao GX (2005) Study of geographical distributional pattern of the subfamily Hormaphidinae (Homoptera, Hormaphididae). Acta Zootaxonomica Sinica 30: 252–256.

Heie OE (1980) The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. I. General Part. The Families Mindaridae, Hormaphididae, Thelaxidae, Anoeciidae, and Pemphigidae. Scandinavian Science Press Ltd., Klampenborg, 236 pp.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. doi:10.2307/2410846

Normark BB (1996) Phylogeny and evolution of parthenogenetic weevils of the *Aramigus tessellatus* species complex (Coleoptera: Curculionidae: Naupactini): Evidence from mitochondrial DNA sequences. Evolution 50: 734–745. http://www.jstor.org/stable/pdfplus/2410846.pdf [accessed 21.III.2011] doi:10.2307/2410846

Ortiz-Rivas B, Martínez-Torres D (2010) Combination of molecular data support the existence of three main lineages in the phylogeny of aphids (Hemiptera: Aphididae) and the basal position of the subfamily Lachninae. Molecular Phylogenetics and Evolution 55: 305–317. doi:10.1016/j.ympev.2009.12.005

Pergande T (1901) The life history of two species of plant-lice inhabiting both the witch-hazel and birch. United States Department of Agriculture, Division of Entomology, Technical Series 9: 1–44.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817–818. doi:10.1093/bioinformatics/14.9.817

Qiao GX, Zhang GX (2004) Record of *Hormaphis* Osten-Sacken (Aphididae: Hormaphidinae) from China, with description of a new species. Oriental Insects 38: 277–282.

Remaudière G, Remaudière M (1997) Catalogue of the World's Aphididae. Institut National de la Recherche Agronomique, Paris, 473 pp.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. doi:10.1093/bioinformatics/btg180

Stern DL (1994) A phylogenetic analysis of soldier evolution in the aphid family Hormaphididae. Proceedings of the Royal Society of London Series B: Biological Sciences 256: 203–209. http://www.jstor.org/stable/pdfplus/50356.pdf [accessed 21.III.2011] doi:10.1098/rspb.1994.0071

Swofford DL (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596–1599. doi:10.1093/molbev/msm092

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882. doi:10.1093/nar/25.24.4876

von Dohlen CD, Gill DE (1989) Geographic variation and evolution in the life cycle of the witch-hazel leaf gall aphid, *Hormaphis hamamelidis*. Oecologia 78: 165–175. doi:10.1007/BF00377152

von Dohlen CD, Kurosu U, Aoki S (2002) Phylogenetics and evolution of the eastern Asian–eastern North American disjunct aphid tribe, Hormaphidini (Hemiptera: Aphididae). Molecular Phylogenetics and Evolution 23: 257–267. doi:10.1016/S1055-7903(02)00025-8

von Dohlen CD, Moran NA (2000) Molecular data support a rapid radiation of aphids in the Cretaceous and multiple origins of host alternation. Biological Journal of the Linnean Society 71: 689–717. doi:10.1006/bijl.2000.0470

von Dohlen CD, Stoetzel MB (1991) Separation and redescription of *Hormaphis hamamelidis* (Fitch 1851) and *Hormaphis cornu* (Shimer 1867) (Homoptera: Aphididae) on witch-hazel in the eastern United States. Proceedings of the Entomological Society of Washington 93: 533–548. http://biostor.org/cache/pdf/30/f8/1c/30f81c9b6406e63f5df75ad2c1d9ff87.pdf [accessed 21.III.2011]

Zhang DX, Hewitt GM (1996) Nuclear integrations: challenges for mitochondrial DNA markers. Trends in Ecology & Evolution 11: 247–251. doi:10.1016/0169-5347(96)10031-8

Zhang ZY, Lu AM (1995) Hamamelidaceae: geographic distribution, fossil history and origin. Acta Phytotaxonomica Sinica 33: 313–339.